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Letter to the Editor: Acute Basal Ganglia Necrosis Associated With Cytarabine Therapy

To the Editor: Cytarabine has been recognized as an important drug in the treatment of acute leukemia over the last two decades [1]. High-dose schedules carry a well-known risk of various neurologic toxicities, of which the acute cerebellar syndrome is the most common [2]. We encountered isolated acute basal ganglia necrosis associated with cytarabine therapy, which we believe to be the first reported instance. It occurred in a 35-month-old boy who was treated in October 1995 for combined bone marrow and testicular relapse of acute lymphoblastic leukemia (ALL) without central nervous system (CNS) involvement. Neurologic examination was normal at that time. On the third day of the second course of salvage therapy using the VANDA protocol [dexamethasone (20 mg/m^2 orally on days 1–5), cytarabine (1,000 mg/m^2 intravenously every 12 h on days 1 and 2), etoposide (150 mg/m^2 intravenously on days 3–5), mitoxantrone (8 mg/m^2 intravenously on days 3 and 4), and asparaginase (10,000 U/m^2 intravenously on days 7, 9, 11, and 13), plus intrathecal cytarabine (20 mg), methotrexate (8 mg), and prednisone (6 mg) on day 5], the patient suddenly deteriorated neurologically. He became lethargic, hypotonic, and mute. Cerebellar examination was normal. Mild horizontal nystagmus was present on lateral gaze. Cranial nerves were intact in function. Deep-tendon reflexes were present, but weak. Serum and CSF chemistries were normal. Centrifugation and staining for malignant cells were negative. Cerebral CT scan was normal. Cranial MRI on day 5 showed abnormal signal intensity involving primarily gray matter of the basal ganglia. Over the next several days, his neurologic status improved gradually, but the patient developed clinical features of secondary parkinsonism. By day 21, cerebral MRI showed features compatible with basal ganglia necrosis. Two years later, his neurologic status has steadily improved. There is no cognitive impairment. Cerebral MRI shows unchanged findings without cerebellar atrophy.

Basal ganglia necrosis is rare in children. The main causes are toxic (carbon monoxide, methanol, or cyanide), hypoxo-ischemic, metabolic (sulfite oxydase deficiency), or postinfectious (e.g., measles, mycoplasma pneumoniae) [3]. Neuroleptic-induced persistent parkinsonism has also been associated with putaminal hypointensity in young patients [4].

We are aware of one previous report of parkinsonism in a child associated with cytarabine therapy [3]. Cranial MRI revealed signal intensity involving the gray matter of the basal ganglia, adjacent temporal and frontal lobes, and cerebellum. Our patient did not present any of the risk factors associated with cytarabine-induced CNS toxicity: age, abnormal renal or hepatic function, or prior central nervous system involvement. The total dose of cytarabine, which appears for several authors to be the chief cause of neurologic toxicity, was low (8 g/m^2) [2]. The importance of the cumulative dose of cytarabine as a factor has been suggested, but there has been great variation from case to case in the dose required to induce neurologic complication [5]. In our patient (cumulative dose from the beginning of the treatment, 17.8 g), we cannot rule out that there was subclinical damage after the first course of cytarabine that became clinically apparent only after the second course. Thus, a careful review of all the etiologies, the previous similar report in a child, and the temporal relationship between the administration of cytarabine and the onset of neurologic symptoms support the probable etiologic role of cytarabine or its metabolites as the cause of this neurologic toxicity. We must underline that the CNS toxicity in our patient is restricted to the basal ganglia, without cerebellar toxicity or atrophy of the vermis.

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Letter to the Editor: Experience Treating a Patient With Bloom Syndrome and Acute Myelogenous Leukemia

To the Editor: Bloom syndrome is an autosomal recessive disorder of genomic instability and predisposition to malignant tumors. Its characteristics are severe prenatal and postnatal growth retardation, variable sun-sensitive telangiectatic facial erythema, and a narrow face [1]. Bloom syndrome leads to malignant tumors in about 25% of patients [2], frequently at more than one primary site [3]. Therapy of malignant disorders in these rare patients is hampered by extremely poor tolerance for cytostatic drugs. Our detailed experience in treating such a patient may be helpful to others faced with this difficult problem.

In our female patient, Bloom syndrome was diagnosed at age 4 years. (See ref. 4 for details.) When 15, acute myelo-monocytic leukemia (FAB-type M4) was diagnosed; blood count showed Hb of 6.5 g/dl, WBC of 39,700/mm^3 with 86% blasts, and 33,000 platelets/mm^3. Bone marrow smear revealed 80% monoblasts (80% peroxidase-positive, 50% esterase-positive, 70% of monocytic immunophenotype). We tried to induce a remission with multiagent chemotherapy, using the consolidation regimen of the current German Acute Myelogenous Leukemia (AML) treatment study (AML-BFM-93). The doses for cytarabine and doxorubicin were reduced to 50% (Table I). The first block of chemotherapy was interrupted on day 24 due to fever (39.8°C). Under i.v. antibiotic and antimycotic treatment the patient developed a bartholinitis. Marsupialization led to defervescence and relief of local symptoms. Bone marrow biopsy on day 41 revealed reduced cellularity with persistence of blasts (86%). A second course of chemotherapy was started on day 42 after admission (Table I). Severe mucositis and massive gastrointestinal bleeding (day 53) required interruption of chemotherapy. During the next 2 weeks the patient lost 5 kg of weight, and was periodically febrile with episodes of tachycardia and tachydyspnea. Blood pressure remained stable. Due to recurrent gastrointestinal bleeding and hematopoietic insufficiency, 14 units of red blood cells and 72 units of platelets were given. In view of the poor tolerance of cytostatic treatment and the inadequate results, further therapeutic attempts were restricted to symptom control on an outpatient basis. At home she remained stable and did fairly well for a month. She finally developed pneumonia. At this time she had a WBC of 14,000/mm^3 with 80% blasts. She died from respiratory failure. An autopsy was not done.

TABLE I. Chemotherapy Administered for Acute Myelogenous Leukemia to a 15-Year-Old-Girl With Bloom Syndrome*

	Drug	Dose in mg/m^2	Route	Day
Block 1	Cytarabine	45	i.th.	7
	Prednisone	40	p.o.	1–15
	Thioguanine	60	p.o.	1–15
	Doxorubicin	17	i.v.	3, 10
	Vincristine	1.5	i.v.	3, 10
	Cytarabine	39	i.v.	16–19
Block 2	Cytarabine	45	i.th.	9
	Prednisone	40	p.o.	1–16
	Thioguanine	60	p.o.	3–16
	Doxorubicin	22	i.v.	8
	Vincristine	1.5	i.v.	8
	Cytarabine	51	i.v.	3–16
				10, 11, 14

*Block 1 was started on day 12, and block 2 on day 42 after admission. p.o., per os; i.v., intravenous injection; i.th., intrathecal injection.

Poor tolerance of cytostatic treatment is known in patients with Bloom syndrome. However, some of them have successfully been treated, partly with standard drug doses, for acute lymphoblastic leukemia (ALL) [2,5], non-Hodgkin lymphoma of B-cell type (B-NHL) [6] or

TABLE II. Cumulative Doses of Cytostatic Agents Given to 2 Patients With Bloom Syndrome Within 8 Weeks of Treatment for Malignancy*

Drug (mg/m^2)	Patient Y.M. (reference [6]), B-non-Hodgkin lymphoma	Our patient, acute myeloid leukemia FAB M4
Doxorubicin	60	56
Vincristine	1.5	4.5
Cytarabine	800	603
Thioguanine		1,640
Cyclophospamide	2,200	
Methotrexate	3,000	
Etoposide	1,200	
Outcome	Treatment continued, long-term remission	Treatment discontinued, no remission, death

*Corticosteroids omitted.

unclassified [7], and for nephroblastoma [8]. By contrast, all reported attempts to induce remission of AML in patients with Bloom syndrome have failed, as did ours [2], perhaps because drug doses had to be reduced. In contrast to ALL, NHL, or solid tumors, treatment of AML requires intensive chemotherapy to achieve remission, and this almost always lead to severe bone marrow aplasia [9].

It is true that a Japanese girl with Bloom syndrome and B-NHL tolerated higher cumulative doses [6] than did our patient (see Table II), a fact that might be explained by interindividual differences between patients or by different drug scheduling. Our AML patient received daily oral thioguanine continuously over 14 days, while the Japanese B-NHL protocol consisted of intravenous applications of drugs on 5–6 out of 14–16 days. Spreading the drug doses over time possibly augments side effects in Bloom syndrome.

In conclusion, successful therapy of AML in patients with Bloom syndrome has so far been prevented by the discrepancy between the treatment intensity needed to induce remission, and poor tolerance of these patients for cytostatic drugs.

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Letter to the Editor: Chemotherapy for Spinal Cord Astrocytoma

To the Editor: We are writing in response to the case report by Bouffet et al. [1] regarding treatment of a spinal cord astrocytoma with carboplatin and vincristine. They present a 30-year-old woman whose spinal astrocytoma showed a dramatic response to these chemotherapeutic agents. At the end of their report, they say that further reports of such therapy in spinal cord astrocytomas may help clarify whether chemotherapy has a role in the management of these tumors. We therefore thought it pertinent to share an adverse experience with carboplatin and vincristine in a child with a spinal cord astrocytoma.

He presented in 1995, when he was 8 years old, with back pain and stiffness. MRI showed a midline tumor extending from C7 to T4, with an extensive syrinx rostrally and caudally. A radical subtotal operation was performed in May 1995. Postoperative MRI scan showed a small amount of residual tumor, which was less than 5% of the original mass. Institutional pathology was reported as low-grade astrocytoma. He remained neurologically normal postoperatively. Serial MRI scans obtained regularly from May 1995 through August 1996 showed no progression of the tumor or syrinx, and he developed no new neurological signs. He did have progressive kyphoscoliosis. In December 1996, there was evidence of progression on a routine MRI scan. Reoperation and alternatives to reoperation were discussed with the parents. They sought second opinions and then expressed a preference for chemotherapy. Due to the time taken to get second opinions, chemotherapy with carboplatin and vincristine did not begin until February 1997.

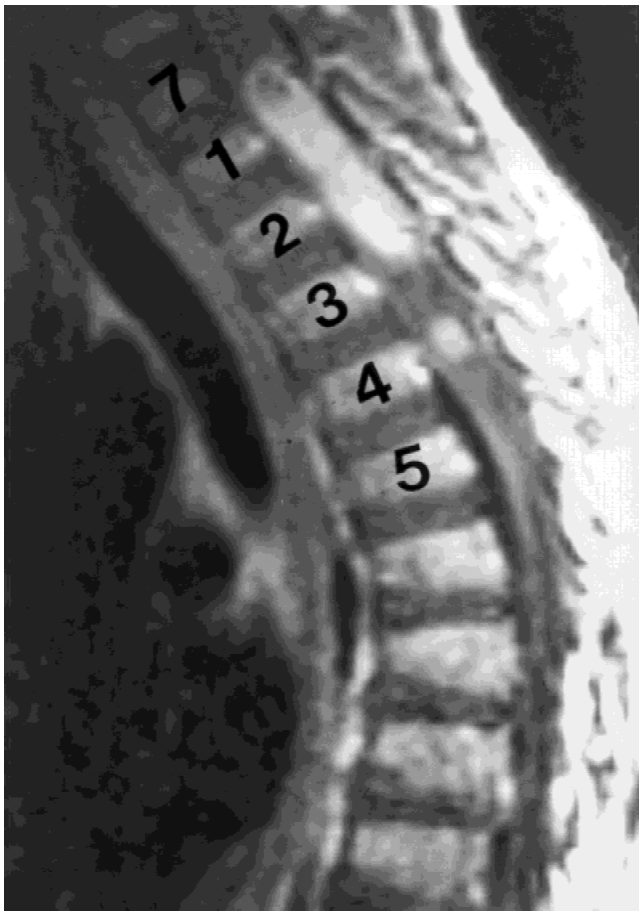

Fig. 1. MRI, performed March 5, 1997, showing tumor at the cervical thoracic junction. Vertebral bodies are numbered.

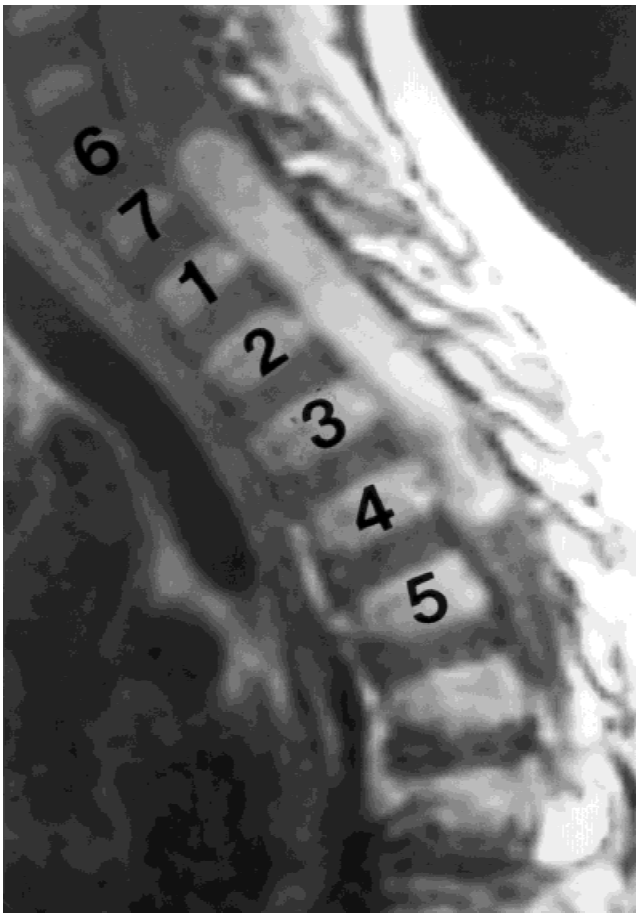

Fig. 2. MRI, performed April 15, 1997, showing an increase in tumor volume compared to Figure 1. Vertebral bodies are numbered.

A repeat scan on March 5, 1997 (Fig. 1) suggested tumor progression compared to the MRI from December 1996. Given that chemotherapy had only begun the previous month, a decision was made to continue the carboplatin and vincristine and scan him in a short period of time. Over the next few weeks, he developed backache and complained of abnormal sensation in the lower limb

and difficulty in initiating urine flow. A repeat scan was done on April 15, 1997 (Fig. 2) which, compared to the scan a month earlier, showed a significant increase in overall volume, with the tumor extending from C7 to T4, and a marked extension of a syrinx. He underwent a radical subtotal resection. Pathology remained pilocytic astrocytoma. He subsequently had radiotherapy.

Carboplatin and vincristine will benefit some patients with spinal cord astrocytomas, but rapid progression may occur on therapy, as seen in our patient. Long-term follow-up has confirmed the efficacy of carboplatin and vincristine for intracranial low-grade astrocytomas [2]. There is a need for the effectiveness of chemotherapy in spinal cord astrocytomas to be explored by cooperative groups.

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Reply

To the Editor: Dr. Foreman et al. express concern about the effectiveness of chemotherapy in spinal cord astrocytoma and report an adverse experience highlighting the limitations of this treatment modality. The aim of our report was to point out the potential for chemotherapy in a disease where chemotherapy has always been neglected. This may be due to the rarity of this disease. Another explanation might be that pediatric neuro-oncologists are often excluded from the decision-making process for postoperative management. It is our duty as neuro-oncologists to assess the role of chemotherapy in common and less common diseases, and the management of spinal cord astrocytoma may benefit from cooperative studies.

The first reports on chemotherapy in low-grade astrocytoma were anecdotal [1], and it took more than a decade to see the development of cooperative protocols which have confirmed the potential for chemotherapy in this group of tumors [2]. We hope that enough favorable anecdotal reports—should they be forthcoming—will provide enough justification to porceed to a cooperative study of spinal cord astrocytoma. Since our first report, 2 more children have been successfully treated in our institution. We encourage the accrual of information from successful or adverse experiences.

Before designing cooperative protocols in this area, however, practical questions need to be discussed. The choice of the best drug combination is obviously important. Is there a need to consider different protocols for low-grade and high-grade astrocytoma of the spine? The timing of chemotherapy is another important issue. In their report, Foreman et al. used chemotherapy at the time of relapse. However, residual tumor was present after the first operation, and this raises the question of adjuvant chemotherapy for incompletely resected tumors. It is well-known that low-grade astrocytomas can exhibit widely variable growth rates, some residues remaining quiescent for years without specific therapy. The natural history of pediatric spinal cord astrocytoma is still unclear, and the risk of progression for patients with subtotal resection needs to be defined.

Most reports have come from single institutions, and the first step towards a cooperative trial might be to set up a data center in order to improve our knowledge from this as yet poorly explored group of diseases.

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Letter to the Editor: The Importance of Molecular Screening of 11q23 Abnormalities in Childhood Acute Lymphoblastic Leukemia: Has the t(11;19)(q23;p13) a Higher Frequency Than That Revealed by Conventional Cytogenetic Techniques?

To the Editor: We have read with interest the report by Ida et al. [1] on the use of reverse transcriptase-polymerase chain reaction (RT-PCR) to detect 11q23 abnormalities that produce rearrangement of the MLL gene. We consider it valuable to carry out a molecular study on diagnosis of all cases of childhood acute lymphoblastic leukaemia (ALL), first with Southern blot and in cases in which rearrangement takes place with PCR, since, as demonstrated here in two cases, these abnormalities can remain undetected by conventional cytogenetic techniques. Their detection can be considered of special importance if one takes into account the poor prognosis of patients with translocations involving chromosome band 11q23 [2] in contrast to deletions and inversions at this band that have a favourable prognosis and lack MLL gene rearrangement [3].

In April 1996, we started a prospective study of paediatric ALL that included analysis of the hybrid genes TEL/AML1, E2A/PBX1, and BCR/ABL by RT-PCR together with a study of the rearrangements of the MLL gene and p16 delections by Southern blot. Until June 1997, we had studied 15 patients with PCR and 11 of these with Southern blot (9 B-precursor ALLs and 3 T-cell ALLs).

For the study of the 11q23 rearrangements, DNA was extracted from bone marrow mononuclear cells, digested with both Bam HI and Bgl II restriction enzymes, and hybridized with the B859 probe, containing MLL exon 5-11 sequences, labelled with P^{32} using the published methods [4].

We obtained 2 patients, 9 and 7 years old, with rearrangement of the MLL gene (Fig. 1) both with a T-cell immunophenotype (the first TdT+, CD2+, CD5+, CD7+, cCD3+, CD1+, CD8+, CD34+, CD10+, CD45+, CD45RO+, CD38+ (34.1%), CD71+, CD21+ (27.6%), CD13+ (32.6%), HLADR−, CD4−, sCD3−, TCR α/β−, CD45RA−, CD19−, CD20−, CD22−, CD14−, CD15−, and the second TdT+, CD2+, CD5+, CD7+, cCD3+, sCD3+, CD1+, CD4+, CD8+, TCR α/β+, CD34+, CD10+, CD45+, CD45RA+, CD45RO+, CD38+, CD71+, CD19−, CD20−, CD22−). In the first case the karyotype was 46,XX,der (19p) and in the second 46,XY,11q-. In the latter case, a t(11;19) was identified by in situ hybridization (FISH).

From these two cases we can deduce that the translocations involving chromosome band 11q23 can remain undetected, as in our former case, if molecular screening is not carried out. Moreover, a quick and simple technique such as PCR is necessary in order to detect the precise molecular lesion, especially when the specific translocation has not been detected by cytogenetic methods. In the second case, this would have occurred if FISH hadn't been performed. Finally, we emphasize the presence of a t(11;19) in one patient and its possible existence in another, who presented the der (19p) chromosome karyotype and rearrangement of the MLL gene in the Southern blot. The t(11;19)(q23;p13) has been described in T-cell ALL but, according to cytogenetic studies, is not common [5]. However, only FISH or mRNA detection of the MLL/ENL gene or, less frequently, of the MLL/ELL gene by PCR [6] can definitively confirm whether a patient presents this translocation. Therefore, these cases suggest that the t(11;19)(q23;p13) is perhaps more common than described by conventional cytogenic techniques.

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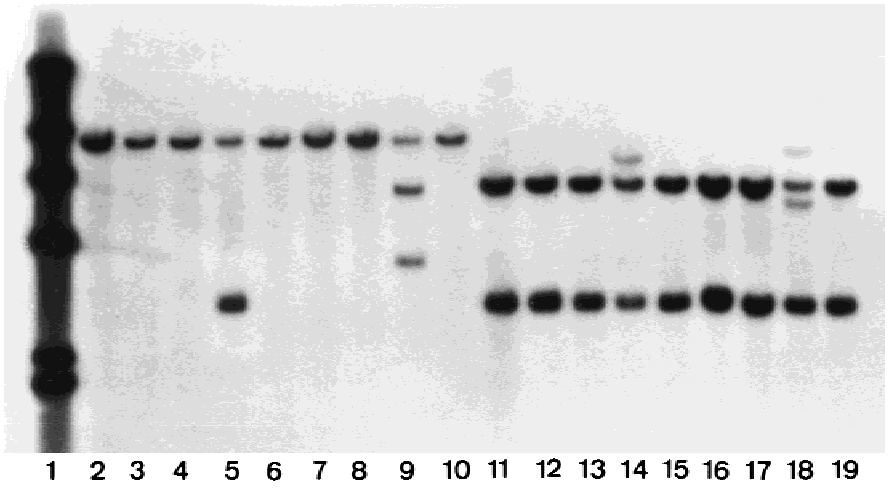

Fig. 1. Filter containing ALL DNAs hybridized with the B859 probe, which explores the MLL breakpoint region at chromosome 11q23. **Lane 1:** Molecular weight marker (lambda phage/Hind III digest labeled with ^{32}P) with bands of 23.1, 9.4, 6.6, 4.4, 2.3, and 2.0 Kb. **Lanes 2–10:** Bam HI digested ALL DNAs. **Lanes 11–19:** DNAs from the same cases digested with Bgl II. Lanes 5 and 14 and 9 and 18, respectively, are the cases described in the text.

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Reply

To the Editor: Anguita et al. reported that t(11;19)(q23;p13) may be more common than described by conventional cytogenetic techniques. We agree with their opinion because we had one acute lymphoblastic leukemia (ALL) patient who had an initially normal karyotype and then had the t(11;19) abnormality at relapse. Interestingly, the *MLL* gene was found to be rearranged at both diagnosis and relapse, suggesting that the leukemic cells of this patient at diagnosis were assumed to have had the t(11;19). It is difficult to analyze the precise breakpoint of the t(11;19) because it is a subtle abnormality [1]. In this regard, *MLL-ENL* and *MLL-ELL/MEN* mRNA were identified in t(11;19)(q23;p13.3) and t(11;19)(q23;p13.1), respectively. The former was found in ALL and acute myeloid leukemia (AML), and the latter only in AML [2].

In 11q23 chromosomal abnormalities other than t(11;19), cytogenetic del(11)(q23) was considered to be t(6;11)(q27;q23) by fluorescence in situ hybridization (FISH) [3] or reverse transcriptase-polymerase chain reaction (RT-PCR) [4]. The t(9;11) is also difficult to analyze cytogenetically, and is often determined as being of normal karyotype by conventional G- or Q-banding methods [5]. One of the 4 patients whose karyotypes were unsuccessfully analyzed was identified to be t(9;11) by RT-PCR in our study [6]. In infant leukemia, the frequency of *MLL* gene rearrangements was reported to be approximately 80%, higher than the *MLL* gene rearrangement found by cytogenic analysis (50–60%) in our study [5]. ALL infants with the *MLL* gene rearrangement had a significantly poor clinical outcome [5,7,8].

These results combined with those in the literature suggest that t(11;19) as well as t(6;11) and t(9;11) are difficult to identify cytogenetically if good metaphases are not obtained. Recently we reported that *MLL-CBP* and *MLL-p300* were involved in t(11;16) and t(11;22) therapy-related leukemia [9,10]. These abnormalities were also difficult to identify cytogenetically. Patients with *MLL* gene rearrangement had a poor prognosis in childhood ALL, regardless of presenting age [11]. Notably, Behm et al. [12] reported that cytogenetics did not detect an 11q23 abnormality in 13 (33%) of 39 childhood AML patients with *MLL* gene rearrangements. Not only ALL patients, as suggested by Anguita et al., but also AML children should be examined for *MLL* rearrangements by Southern blotting first, and by either FISH or RT-PCR to predict clinical outcome.

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Letter to the Editor: Bone Marrow Biopsy as Prognostic Indicator in Childhood Acute Lymphoblastic Leukemia—Another Opinion

To the Editor: In a recent issue of this journal, Schultz et al. retrospectively evaluated the day 7 bone marrow (BM) biopsy as a prognostic measure of outcome in 88 children with acute lymphoblastic leukemia (ALL) enrolled in five different CCG protocols and treated at their institution [1]. The authors concluded that the information gained from the day 7 BM biopsy can improve prediction of outcome in children with ALL but also that a prospective confirmation with larger studies is needed.

BM biopsy, however, as acknowledged by Schultz et al. [1], basically remains an invasive procedure requiring ''conscious sedation or general anesthetic.'' In addition, BM biopsy reproducibility is hampered by sampling error, inadequate yield, and variability of evaluation, according to the expertise of the pathologist. Laboratory time and costs are also relatively high, making this technique neither easily available nor recommendable for large cooperative studies.

Also to be considered is that the lowest disease-free survival (DFS) found after the patients' stratification based on the ABI-aspirate value $</\geqslant .06$ was 51%. This information was obtained in a retrospective study and on a limited number of patients, who underwent different types of treatment. These data may not be especially useful, since it is possible to recognize subsets of patients with DFS even much lower than 51% simply by using white blood cell count and age [2], steroid response (alone or with additional features), and marrow residual blast infiltration after 7 or 14 days of treatment or delay in achieving complete remission [3–5].

More sophisticated techniques such as the polymerase chain reaction may also allow the detection of minimal residual disease (MRD) and the early recognition of patients at very high risk of relapse [6]. Detection of MRD at the beginning of maintenance therapy in T-cell ALL has been very recently reported to predict virtually all relapses in this subset of patients [7].

Results in childhood ALL can vary greatly, depending on the choice of stratification criteria and treatment modalities. Simplification in the stratification of patients and in the report of results is thus considered important for a better understanding of overall results in clinical trials [2]. It is generally acknowledged that a prognostic factor, to be clinically useful, should be feasible, reproducible, specific, sensitive, and widely available, especially in the context of national or international cooperative trials. The addition of any new prognostic factors to those already available should thus be carefully evaluated before incorporation into front-line clinical trials.

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Reply

To the Editor: We appreciate the amount of attention that Dr. Rizzari has given to our recent article entitled ''Importance of the Day 7 Bone Marrow Biopsy as a Prognostic Measure of the Outcome in Children With Acute Lymphoblastic Leukemia'' [1]. Dr. Rizzari makes 6 points we will address.

His first area of concern is that a bone marrow biopsy requires either conscious sedation or general anesthetic. We previously had stated that we think both a bone marrow aspirate and biopsy require either conscious sedation or general anesthetic in the pediatric population. Performing both a biopsy and aspirate does not increase the need for this type of sedation, although the duration may be increased by 5 to 10 minutes. In addition, more centers in North America are beginning to use short outpatient general anesthetic, making it even easier to perform the combination of aspirate and biopsy.

The second point is that bone marrow biopsy reproducibility is an issue. Sampling variability is a theoretical problem that applies to all methodologies used to measure early response including bone marrow aspirate, biopsy, and peripheral blood count. Many hematopathologists feel that both a marrow aspirate and biopsy are required to fully assess the status of a marrow. As we stated in our paper [1], 5% of patients had inadequate aspirates, 7% had inadequate biopsies and 2% had both an inadequate aspirate and biopsy. The bone marrow aspirate is an established prognostic indicator for large multicenter studies within the Children's Cancer Group (CCG) in North America. Since the bone marrow biopsy has a similar variability to aspirates, we feel that it is acceptable that the bone marrow biopsy be investigated in large studies in combination with the aspirate as a prognostic factor as a method to potentially improve the predictability of the bone marrow aspirate. The advantage of the biopsy is that accurate assessment of marrow cellularity and leukemic burden can be made. The same assessment of cellularity cannot be made, if a dilute aparticulate aspirate is obtained.

The third point made is that the laboratory cost and time is high. Within our center a bone marrow biopsy is not a costly procedure, but we understand that a bone marrow biopsy can be expensive at other centers. On the other hand, relapse of leukemia is even more expensive. Improved prognostication for patients with ALL in order to alter therapy in patients with a poorer prognosis, is considered acceptable by most pediatric oncologists. This is evidenced by continued evaluations using, cytogentics, RT-PCR, PCR, and clonogenic assays. The costs associated with these complex investigations are not trivial.

The fourth point made by Dr. Rizzari is that the bone marrow cellularity estimated by the biopsy and the aspirate does not identify a sufficiently poor prognostic group to be useful. He states that age and WBC, the steroid response measured by peripheral blast counts after 7 days, the day 7 or 14 bone marrow aspirate, or an induction failure are better prognostic factors. We agree that a day 28 bone marrow aspirate with >5% blast conveys a poor prognosis, but do not consider this to be an early prognosticator. Since we can only evaluate patients treated by CCG protocols using vincristine, prednisone, L-asparaginase ± daunomycin induction, we cannot address the early Prednisone response as a prognostic indicator. Recent evaluations of age and WBC as prognostic indicators for the outcome of patients treated on CCG protocols revealed that these can no longer identify patients whose EFS is <60% when patients with the t(9;22) translocation are excluded [unpublished data]. In fact, cytogenetic indicators appear to be the only factors that can identify patient with a very poor outcome treated on current CCG trials. On the other hand, the day 7 and 14 bone marrow aspirate continues to be able to identify patients who have a 10–30% poorer outcome in slow early responders [2]. Our point was that our preliminary study demonstrated an improved predictive value by the bone marow aspirate when the bone marrow cellularity from the biopsy was also considered [Fig. 3]. To summarize, current CCG protocols have improved patient outcomes and decreased the predictive value of previously published factors such as age and WBC.

Dr. Rizzari's fifth point is that the use of PCR will allow the identification of MRD in patients later in

therapy and will be a more accurate prognostic marker. The limitations of this approach is that evaluations using PCR or RT-PCR for detection of ALL have only been done at the end of induction and not at earlier time points [3,4]. In addition, minimal residual disease measured by these techniques is many times present at the end of induction and their prognostic significance at the end of induction has not been established by large studies. The point of using the day 7 bone marrow aspirate and biopsy is to determine a population of patients who will benefit from an early alteration in therapy with the goal to decrease the evidence of MRD at a later time point. Thus, a day 7 bone marrow aspirate and biopsy detects disease at an early time point and more expensive molecular methods can be reserved to detect disease at later time points or after therapy has finished. In addition, the utility of RT-PCR or PCR detection for MRD and its prognostic value still needs to be determined. An example is that detection of ALL cells expressing p210 BCR/ABL after marrow transplantation does not predict relapse [5].

The last and sixth point made by Dr. Rizzari is that ‘‘ . . . a prognostic factor to be clinically useful should be feasible, reproducible, specific, sensitive, and widely available.’’ We completely agree with Dr. Rizzari. A bone marrow biopsy on day 7 is both feasible and widely available and it appears to increase the specificity and possibly the sensitivity of the day 7 bone marrow aspirate. As we have already stated [1] the value of the day 7 bone marrow biopsy in combination with the aspirate needs to be validated in larger prospective studies. Studies to evaluate in vivo responses to therapy will provide critical insights into disease/host biology and are worthy of serious study.

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